

Toxicity assessment of chlorpyrifos, λ -cyhalothrin and neem extract against *Dysdercus koenigii* with reference to survivorship, fecundity and some biochemical parameters.

¹Masarrat J. Yousuf*, ²Mohammad Attaullah, ³Ishtiaq Anjum, ⁴Sobia Khawaja

^{1,2,4}Department of Zoology, University of Karachi, Karachi-Pakistan (75270)

³Kohat University of Science and Technology, Kohat Pakistan

Abstract—The present study was designed to evaluate the comparative toxicity of Chlorpyrifos, Lambda (λ) Cyhalothrin and Neem extract (a bio pesticide) against the adults of *D. koenigii*. A gradual increase in the mortality was found with the increase of concentration. The calculated LC50 values for Chlorpyrifos, λ -Cyhalothrin and Neem extract were 0.0069%, 0.045% and 0.0787% respectively.

The order of efficacy of the three pesticides in respect of toxicity was: Chlorpyrifos > λ . Cyhalothrin > Neem extract. Effect on fecundity of adult *D. koenigii* was investigated and it is observed that complete egg inhibition occurred at concentrations of 0.0062%, 0.0039% and 0.048% for Chlorpyrifos, λ . Cyhalothrin and Neem extract respectively.

This showed that the three pesticides are same in the order of efficacy regarding toxicity and fecundity. Neem extract was found more effective to decrease the total protein content as compared with Chlorpyrifos and λ -Cyhalothrin. Inhibitory effects on GOT (Glutamate Oxaloacetate Transaminase) enzyme were calculated based on Spectrophotometric analysis and found to be 10.2% (λ -Cyhalothrin) and 4.9% (Neem extract) where as Chlorpyrifos caused an increase in GOT activity up to 1.28% against the survivorship.

The overall efficacy of the three pesticides against the survivorship was in the order of Chlorpyrifos > λ Cyhalothrin > Neem extract. This report covers effectiveness of the given pesticides in respect of various biochemical parameters GOT, Protein content, toxicity, fecundity and survivorship against the adults of *D. Koenigii*.

Keywords—*Dysdercus koenigii*, Toxicity, GOT, Fecundity.

I. INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important cash crop of Pakistan. Because of its significance of foreign exchange earning in the country, it is known as “white gold”. Pakistan is the fourth largest cotton producer in the world, the third largest exporter of raw cotton in the world and the fifth largest consumer (Ahmad et al., 2011). Pakistan ranks fourth in the production of cotton after China, USA and India (Anonymous, 2007). It is grown in Pakistan on an area of about 3031.5 thousand hectares producing 12452.5 thousand bales of cotton lint and an average yield of 699kg/ha (Anonymous, 2010). This per hectare yield is very low as compared with other cotton producing countries of the world. One of the major reasons for the low yield is insect pest infestation.

The insect pest's spectrum of cotton is very broad and almost 1326 species of insect pests attack this crop throughout the world, however main losses are because of its susceptibility to about 162 species of insect pests (Ahmad et al., 2011). Because of the wide range of insects that attack cotton, the crop has been identified as the largest worldwide consumer of insecticides. Cotton accounts almost 2.4% of the total cultivated acreage worldwide, it consumes approximately over 25% of the pesticides (Krattigar, 1997). *Dysdercus* species are known by the trivial names of Cotton stainer bug, Red cotton stainer, Red seed bug of Malvaceae and Red cotton bug in Pakistan.

It belongs to order Hemiptera, family Pyrrhocoridae which is a cosmopolitan family of Pentatomomorphus bugs consisting of about 65 genera and 400 species (Schaefer and Ahmad, 2000). These bugs have prominent mouth parts which are adopted for piercing and sucking. Bugs manifestation cause huge losses annually to the fruits, vegetables and crops globally. Mealy bug *Phenacoccus solenopsis* besides cotton causes devastations to many other economic crops such as vegetables, ornamental plants and has been reported infesting 149 plant species (Afzal et al., 2009).

Dysdercus koenigii is a pest loving plant, considered as a minor pest of cotton. Medium to large sized nymphs and adults feed on seeds within developing cotton bolls (Sprenkel, 2000). They feed both on immature and mature seeds. The name cotton stainer is derived from their habit of piercing the bolls and thereby contaminating them with the fungus *Nematospora* which stains the fibers (Pearson, 1958 and Frazar, 1994). *Dysdercus koenigii* is active throughout the year and passes winter in the adult stage. They hatch in about one week, laying about 100-130 pale yellow eggs in sand, debris or decaying vegetable matter in spring season. There are five nymphal instars each requires about 21-35 days to complete (Sprenkel, 2000).

Dysdercus koenigii, like other insect pests has been controlled by various chemical and non-chemical means globally. The use of biopesticides is safe to natural enemies and their integration with natural enemies has a good impact on crop yield parameters.

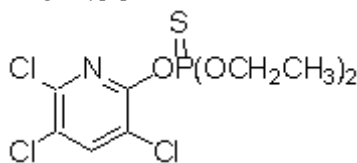
Neem, a botanical insecticide, as compared with all other chemical and biopesticides has the beauty that it possesses a wealth of scientific studies, numerous international conferences and several major volumes of information (Koul and Wahab, 2004). The complex triterpenoid, azadirachtin, obtained from the seeds of the neem tree *Azadirachta indica* is a potential insect growth regulator and feeding deterrent with minimal mammalian toxicity and environmental persistence. Main chemical broadside of Neem extract is a mixture of 3 or 4 related compounds belonging to a general class of natural product called “triterpenes”, more specifically “limonoids”. Azadirachtin, Salanin, Meliantriol and Nimbin are the best known limonoids. Azadirachtin is structurally similar to insect hormone “ecdysone” which controls the process of metamorphosis. Neem extract is the most commonly used botanical biopesticide with a narrow target range and a very specific mode of action. They are slow acting and suppress rather than eliminate a pest population. Neem products are safer to humans and the environment than conventional pesticides and present no residue problems.

Neem is less toxic to natural enemies than commercial insecticides (Ranga Rao, 2008; Roger et al., 2009; Sahito et al., 2011; Shabozoi et al., 2011).

Kodandaram *et al.* (2008) assessed the toxicity of various botanicals against third instar nymphs of *D. koenigii* and found dose dependent changes in metamorphosis and mortality. *D. koenigii* can be controlled by the application of multineem and imidacloprid (Fakhri et al., 2011).

Neem extract is used by many researchers to control insect pests (Tabassum et al., 1992, 1994, 1998; Naqvi and Aslam 2000; Prenachandra et al., 2005; Garcia et al., 2006 and Nathan et al., 2007). Chlorpyrifos is an organophosphate insecticide which is effective in controlling a variety of insects, including cut worms, corn worms, cockroaches, grubs, fleas, beetles, termites, fire ants and lice (US EPA 1986). It is used as an insecticide on grain, cotton field, fruits, nuts and vegetable crops and as well as on lawns and ornamental plants (Berg, 1986). Insecticidal activity of Chlorpyrifos is caused by the inhibition of the enzyme acetylcholinesterase, which result in the accumulation of the neurotransmitter- acetylcholine, at the nerve ending. This results in excessive transmission of nerve impulses, which cause mortality in the target pest. Chlorpyrifos can be used as an emulsifiable concentrate, dust, flow able pellets, spray; granular and wettable powder formulation (Meister, 1992). It acts primarily on pests as a contact poison with some action as a stomach poison.

Toxicity of Chlorpyrifos is found to be different for workers and soldiers of *Captotermes formosanus* (Gatti et al., 2002). Chlorpyrifos at 0.1 µg/L reduces the *Chironomas tentans* survivorship by 67% after 20 days exposure (Rakotondravelo et al., 2006). In laboratory, Chlorpyrifos has been proved to be the best insecticide for the control of mealy bug, *Phenacoccus gossypiphilus* (Saeed et al., 2007).

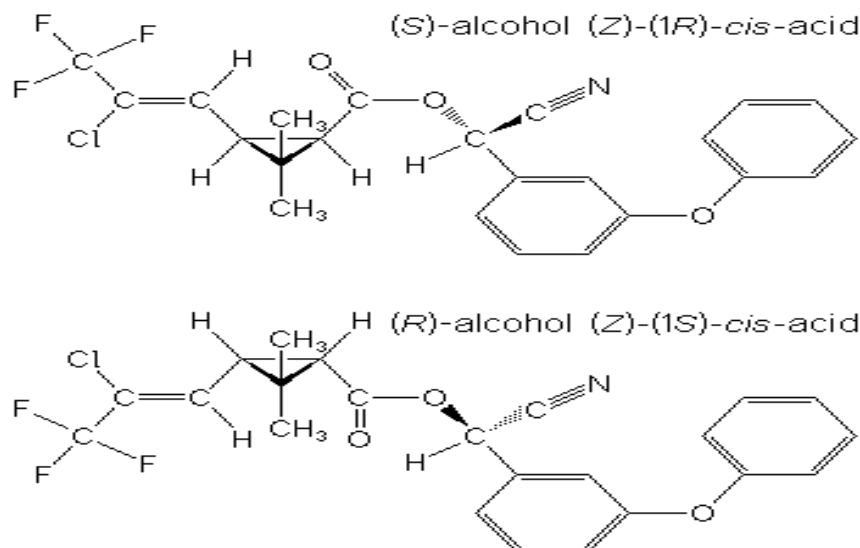


Molecular Structure of Chlorpyrifos

λ-Cyhalothrin is a pyrethroid insecticide registered by the US EPA in 1988. It is a synthetic pyrethroid used to control a variety of pests including aphids, Colorado beetles and butterfly larvae (Kidds and James, 1991). Pyrethroids affect the NS of an organism by disrupting the Na-channels that are involved in the generation and conduction of nerve impulses. It causes rapid paralysis and death to an insect when ingested or exposed externally.

λ-Cyhalothrin has proved one of the controlling insecticide in a number of investigations (Al-Deeb et al., 2001; Arias et al., 2003; Vandekerckhove and Declercq 2004; Snodgrass et al., 2005; Ali and Masarrat 2005-unpublished M.Sc. thesis).

λ-Cyhalothrin has been reported to be effective for mosquito control (Weathersbee et al., 1991). It is highly toxic when ingested.



Molecular Structure of λ -Cyhalothrin

The present investigation showed the comparative toxicity of Chlorpyrifos, λ -Cyhalothrin and Neem extract on the adults of *D. koenigii*. The survivorship data throws light on the comparative toxicity of these chemicals. Effects on various biochemical parameters GOT and protein contents are tested and the comparative analyses on fecundity are carried out thoroughly.

II. MATERIALS AND METHODS

The experimental insect i.e., adults of *Dysdercus koenigii* were collected from plants belonging to Malvaceae and Poaceae families along with their actual food at Karachi University.

Preparation of the Tested Compounds:

The first tested pesticide was Chlorpyrifos (40 E.C). By mixing of 0.0625ml of chlorpyrifos with 99.937 ml of distilled water, a stock solution of 0.0623% was prepared. Further dilutions were prepared by mixing 10ml of 0.0625% concentration with 10ml of distilled water to give 0.03125% concentration. Subsequently, in the same way 0.0078125% and 0.0039% concentration were prepared by using 10ml pipette, clean and neat to make an accurate concentration.

The second tested compound was λ -Cyhalothrin in (2.5 E.C.). A stock of 0.2% was prepared by mixing 99.8ml of distilled water with 0.2ml of λ -Cyhalothrin. Then serial dilution of 0.1%, 0.05%, 0.025%, 0.0125% and 0.00625% concentrations were prepared in the same manner.

The third tested compound was Neem extract (BIOSAL-A). This was obtained from H.E.J. research institute of Chemistry, University of Karachi. It is insoluble in water hence for the preparation of 50% stock solution, Methanol of 50% was mixed with 50ml Neem extract. Further dilution of 12.5% was prepared by mixing of 10ml of 50% Stock solution with 30ml of Methanol. For 3.125%, 10ml of 12.5% concentration was taken and mixed with 30ml of Methanol. In the same way 0.78125%, 0.1953% and 0.0488% concentrations were prepared subsequently. The main chemical constituents of Neem extract (BIOSAL-A) are: Azadirachtin (0.32%), Salanin (1.06%), Nimbin (0.75%), Deacetylnimbin (0.31%), other terpenoids (1.5%) and Neem oil (7.5%).

Method of Treatment

Contact method was used for the determination of relative toxicities of the said insecticides against the adults of *D. koenigii*. Glass bottles of 15cm length were used into which 1ml for all of the three pesticides in their different concentrations were applied. About 10 adults of *D. koenigii* were released in each glass bottle having 10gm of food with 1ml of pesticide i.e. Chlorpyrifos, λ -Cyhalothrin and Neem extract. A control batch of 10 untreated adults was also kept for the determination of environmental effects. Mortality was determined after every 24 hours.

Determination of Fecundity:

Twenty pairs of adults (10 ♂ and 10 ♀) were taken in a glass jar along with their food. They were treated with sub-lethal concentrations of Chlorpyrifos, λ -Cyhalothrin and Neem extract i.e., 0.0039%, 0.00625% and 0.0488% respectively. A control batch of 20 pairs of pesticides was also kept along with each pesticide for comparison. The adults were later on observed for mating and egg laying activity.

Assessment of Survivorship:

Twenty pairs of insect were taken in a glass jar along with their food and applied with sub-lethal concentrations of the said insecticides. A control batch of 20 pairs of adults was also kept with each pesticide for comparison. The adults were later on observed for survivorship.

Protein Assay:

The Biurette method was used to determine the change in the total protein content. 0.5gm of LC50 treated adults were crushed in 2ml of distilled water and homogenized for 2 minutes at 2500rpm. This was then centrifuged at 3500 rpm for 15 minutes. The supernatant was then separated from debris and named as sample (Procedure P-52, enzyme Assay P-52 to 54, summarizes).

III. RESULTS

A gradual increase in the mortality was observed with the increase of concentration. The order of toxicity of the tested pesticides on adult *D. koenigii* was: Chlorpyrifos > λ -Cyhalothrin > Neem extract. (Table I). The calculated LC50 values were 0.0069% (Chlorpyrifos), 0.045% (λ -Cyhalothrin) and 0.07875% (Neem extract) as is evident from Fig. 1, 2 and 3 respectively. This shows the order of efficacy of the said pesticides against adult *D. koenigii*.

Table I: Percent mean mortality of *D. koenigii* adults at various concentrations of Chlorpyrifos, λ -Cyhalothrin and Neem extract.

S. No.	Chlorpyrifos		λ -Cyhalothrin		Neem extract	
	%Conc.	%Mean Mortality	%Conc.	%Mean Mortality	%Conc.	%Mean Mortality
1.	0.0625	90	0.2	80	12.5	94
2.	0.03125	80	0.1	75	3.125	76
3.	0.015625	90	0.05	70	0.78125	68
4.	0.0078125	50	0.025	35	0.1953	58
5.	0.0039	40	0.0125	28	0.0488	50
6.	Control	10	Control	10	Control	06

FIGURES 1-6:

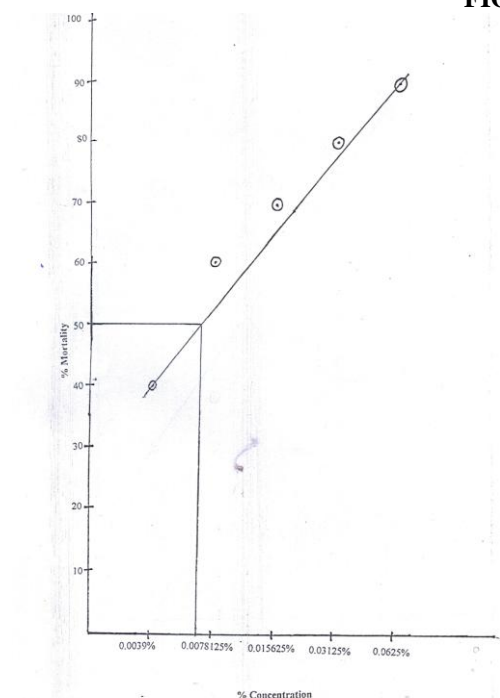


Fig # 1. Linear regression showing LC₅₀ between concentration (Chlorpyrifos) and percent mortality of adult *D. koenigii*

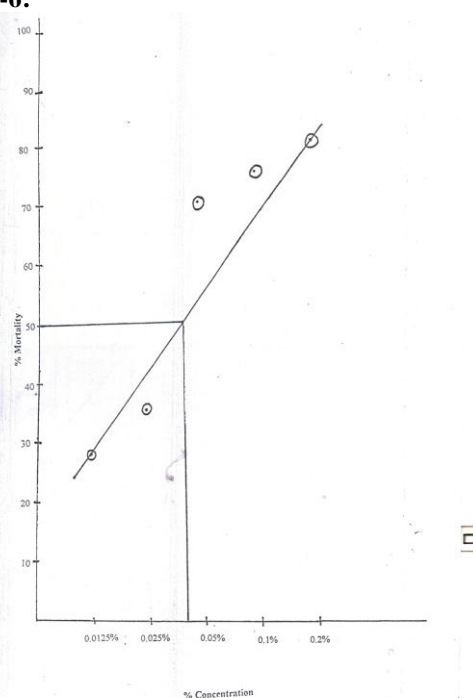
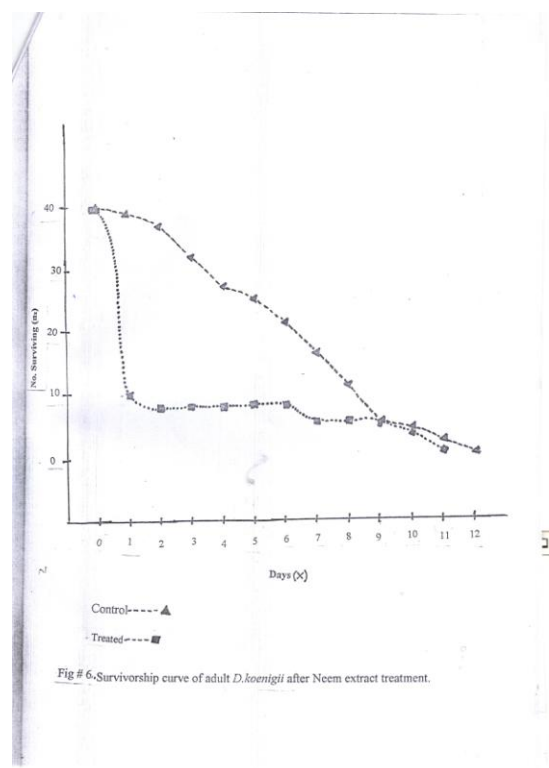
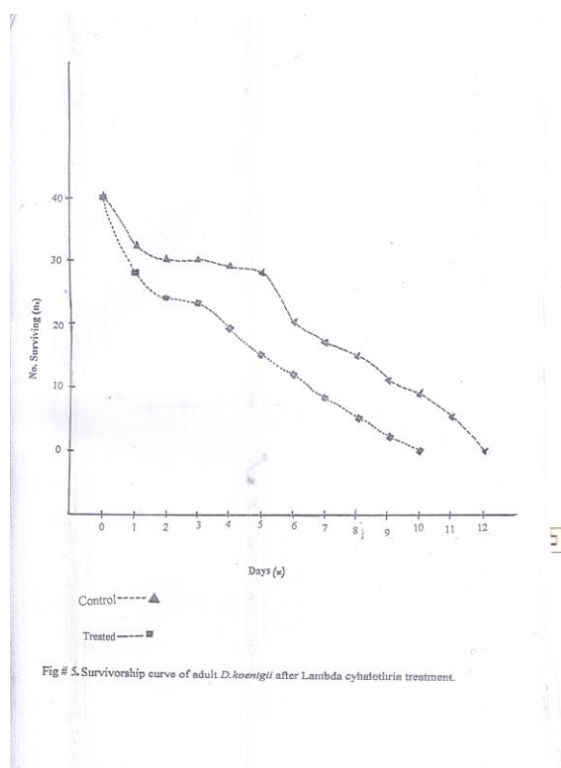
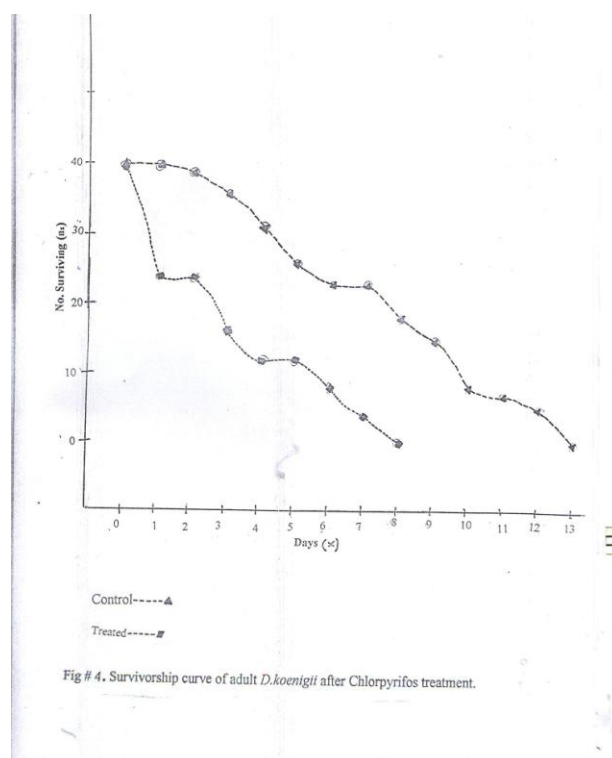
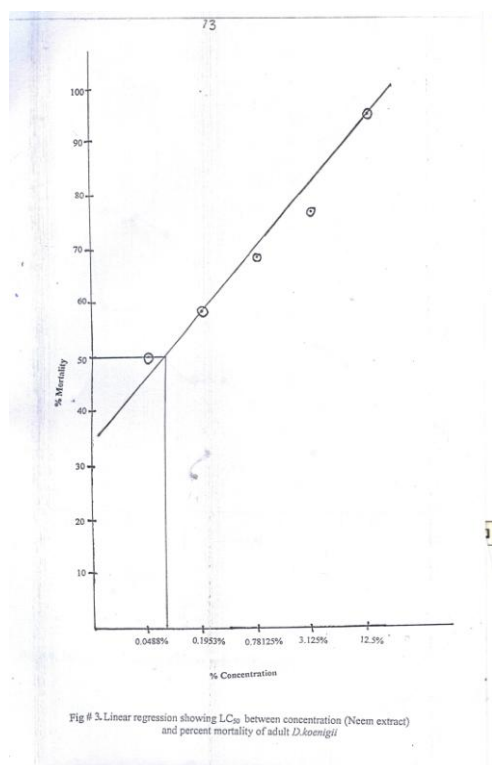


Fig # 2. Linear regression showing LC₅₀ between concentration (Lambda cyhalothrin) and percent mortality of adult *D. koenigii*



The three pesticides were tested for fecundity assessment. Decrease and delay in the numbers of eggs and emergence was observed.

Table II: Effect of Chlorpyrifos, λ -Cyhalothrin and Neem extract on Fecundity of *D. koenigii* adults.

DAYS	CHLORPYRIFOS				λ -CYHALOTHRIN				NEEM EXTRACT			
	No. of eggs		Emergence		No. of eggs		Emergence		No. of eggs		Emergence	
	Cont.*	Treated	Cont.	Treated	Cont.	Treated	Cont.	Treated	Cont.	Treated	Cont.	Treated
1.	Nil	Nil	Nil	Nil	65	Nil	40	Nil	80	Nil	68	Nil
2.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	100	Nil	75	Nil
3.	150	Nil	73	Nil	135	Nil	Nil	Nil	100	Nil	80	Nil
4.	Nil	Nil	Nil	Nil	Nil	Nil	65	Nil	200	Nil	79	Nil
5.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
6.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
7.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
8.	85	Nil	27	Nil	85	Nil	Nil	Nil	Nil	Nil	Nil	Nil
9.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
10.	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	18	Nil	12

Cont*= Control.

Complete egg inhibition occurred at 0.00625%, 0.0039% and 0.048% concentrations of Chlorpyrifos, λ -Cyhalothrin and Neem extract respectively. The order of efficacy was: Chlorpyrifos > λ -Cyhalothrin > Neem extract. An increase in the total protein content occurred after Chlorpyrifos treatment while λ -Cyhalothrin and Neem extract caused a decrease in the protein level (Table III). Neem extract decreased the protein level (7.925 gm/dl) more than Chlorpyrifos, λ -Cyhalothrin (Table III). GOT level was increased to 1.28% after Chlorpyrifos treatment while an inhibition of 10.2% (λ -Cyhalothrin) and 4.9% (Neem extract) was calculated after spectrophotometric analysis (Table III).

Table III: Effects of the tested pesticides on total protein contents and GOT in adults of *D. koenigii*.

S.No.	Pesticides	Total protein level (gr/dl)	GOT	
			%Inhibition	%Activation
1.	Chlorpyrifos	27.249	—	1.28
2.	λ -Cyhalothrin	26.28	10.2	—
3.	Neem extract	18.975	4.9	—

Exposure of the adults of *D. koenigii* for 24 hours to Chlorpyrifos (0.0039%), λ -Cyhalothrin (0.00625%) and Neem extract (0.048%) caused 60%, 70% and 25% decrease in the survivorship respectively, table IV, V and VI.

Table IV: Life table for the adults *D. koenigii* after Chlorpyrifos treatment.

x	nx		lx		dx		qx		ex	
Day	Observed no. of adults alive.		Proportion surviving at start of day x.		No. dying within age interval x to x+1.		Rate of Mortality		Mean Expectation of further life for insects	
	Cont.	Treated	Cont.	Treated	Cont.	Treated	Cont.	Treated	Cont.	Treated
0	40	40	1	1	0	16	0	0.4	7.275	3
1	40	24	1	0.6	1	0	0.025	0	6.275	3.6
2	39	24	0.975	0.6	3	8	0.076	0.33	5.423	2.66
3	36	16	0.9	0.4	5	4	0.138	0.25	4.833	2.75
4	31	12	0.775	0.3	5	0	0.1612	0	4.532	2.5
5	26	12	0.65	0.3	3	4	0.115	0.33	4.307	1.5
6	23	8	0.575	0.2	0	4	0	0.5	3.804	1
7	23	4	0.575	0.1	5	4	0.217	1	2.804	0.5
8	18	Nil	0.45	Nil	3	Nil	0.166	Nil	2.44	Nil
9	15	Nil	0.375	Nil	7	Nil	0.466	Nil	1.833	Nil
10	8	Nil	0.2	Nil	1	Nil	0.125	Nil	2	Nil
11	7	Nil	0.175	Nil	2	Nil	0.285	Nil	1.21	Nil
12	5	Nil	0.125	Nil	5	Nil	1	Nil	0.5	Nil

Table V: Life table for the adults *D. koenigii* after λ -Cyhalothrin treatment.

x	nx		lx		dx		qx		ex	
Day	Observed no. of adults alive.		Proportion surviving at start of day x.		No. dying within age interval x to x+1.		Rate of Mortality		Mean Expectation of further life for insects	
	Cont. Treated		Cont. Treated		Cont. Treated		Cont. Treated		Cont. Treated	
0	40	40	1	1	4	12	0.1	0.3	6.25	3.9
1	36	28	0.9	0.7	6	4	0.166	0.14	6.88	4.357
2	30	24	0.75	0.6	Nil	1	0.1350	0.041	5.96	4
3	30	23	0.75	0.575	1	4	Nil	0.173	4.96	3.15
4	29	19	0.725	0.475	1	4	0.033	0.21	4.12	2.71
5	28	15	0.7	0.375	8	3	0.034	0.2	3.25	2.3
6	20	12	0.5	0.3	3	4	0.285	0.33	3.35	1.75
7	17	8	0.425	0.2	2	3	0.117	0.375	2.85	1.375
8	15	5	0.375	0.125	4	3	0.26	0.6	2.16	0.9
9	11	2	0.275	0.05	2	2	0.181	1	1.77	0.5
10	9	Nil	0.225	Nil	4	Nil	0.44	Nil	1.055	Nil
11	5	Nil	0.125	Nil	5	Nil	1	Nil	0.5	Nil
12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

Table VI: Life table for the adults *D. koenigii* after Neem extract treatment.

x	nx		lx		dx		qx		ex	
Day	Observed no. of adults alive.		Proportion surviving at start of day x.		No. dying within age interval x to x+1.		Rate of Mortality		Mean Expectation of further life for insects	
	Cont. Treated		Cont. Treated		Cont. Treated		Cont. Treated		Cont. Treated	
0	40	40	1	1	1	30	0.025	0.75	5.9	2.275
1	39	10	0.975	0.25	2	2	0.051	0.2	5.038	6.6
2	37	8	0.925	0.2	5	0	0.135	0	4.283	7.125
3	32	8	0.8	0.2	5	0	0.156	0	3.875	6.125
4	27	8	0.625	0.2	2	0	0.074	0	3.5	5.125
5	25	8	0.625	0.2	6	0	0.24	0	2.74	4.125
6	21	8	0.525	0.2	5	3	0.238	0.375	2.166	3.125
7	1	5	0.4	0.125	5	0	0.3125	0	1.6875	3.7
8	11	5	0.275	0.125	6	0	0.545	0	1.22	2.7
9	5	5	0.125	0.125	2	1	0.4	0.2	1.1	1.7
10	3	4	0.075	0.1	3	2	1	0.5	0.5	1
11	Nil	2	Nil	0.05	Nil	2	Nil	1	Nil	0.5
12	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

The remaining population of insects survived only 7 days after Chlorpyrifos exposure, 9 days after λ -Cyhalothrin exposure and 11 days after Neem extract exposure. (Fig. 4, 5 and 6)

The overall nature of the three pesticides showed a significant difference in toxicity and changes caused in various biochemical parameters as is evident from the results.

IV. DISCUSSION

Biological control, cultural control and other non-chemical control measures of pest control are slow acting, less effective and without certainty in respect of required results. Chemical control, although having impaired health risks, is still a fast, efficient and ultimate measure to control the pests and get the required results. To ensure least mammalian toxicity, least environmental pollution, least resistance and persistence problems and more floral production, levels of the controlling agents must be demarcated to control only the target species without threatening rest of the biodiversity.

The LC₅₀ values in the present study for the control of the adults of *D.Koenigii* are determined to be 0.0069% (Cyhalothrin), 0.045% (λ -Cyhalothrin) and 0.07875% (Neem extract). These pesticides can be used within their safety limits to control the said pest with least residue problems.

Neem extract has been used efficiently by various researchers against insect pests (Naqvi et al., 1989, Tabassum et al., 1994). λ-Cyhalothrin and Chlorpyrifos are also used as controlling agents against various pests (Saegraves and Pherson, 2003 and Ralkotondravelo et al., 2006).

Lambda Cyhalothrin, Cypermethrin, Dimilin and Biosal tested against *D.koenigii* has shown the given order of efficacy: Cypermethrin > λ-Cyhalothrin > Dimilin > Biosal. (Aisha Perveen Ph.D. Thesis). Andalin has been tested against *D. koenigii* and found to cause inhibition of adult emergence, delay in metamorphosis, partial wing development and abnormal moulting at 0.1% concentration (Khan and Qamar, 2011). GOT is one of the enzymes, which gives valuable diagnostic information for a number of disease conditions. The enzyme shows sensitivity and changes occur variously by exposing to different pesticides. Increase in GOT activity to 1.28% has been noted when the pest was treated with Chlorpyrifos while a decrease of 10.2% and 4.9% for λ-Cyhalothrin and Neem extract has been observed respectively. The elevation may be due to enzyme induction as a result of Chlorpyrifos stress or it may have affected oxidation by Krebs's cycle.

Transaminases (GOT and GPT) has shown inhibition in activities after the application of pyrethroids (Coopex and Danitol) in stored grain pests, *Tribolium castaneum* and *Sitophilus oryzae* (Masarrat et al., 2007). Proteins are good indicators of metabolic activity of cell. Neem extract was found more effective to decrease the total protein level as compared with λ-Cyhalothrin and chlorpyrifos. Total protein contents in adults of *D. koenigii* are shown to be decreased by 51.649%, 29.74%, 70.16% and 9.72% after the treatment of Cypermethrin, λ-Cyhalothrin, Dimilin and Biosal respectively (Aisha Perveen, Ph.D. Thesis).

This may be due to low level of anabolic activity of cell or higher levels of catabolic activities. The raised levels of GOT also show increase in catabolic activities which is involved in breakdown of proteins to amino acids and routing these amino acids to Krebs's cycle.

Fecundity assessment and survivorship data in the present work shows the relative efficacy of the said pesticides against the adults of *D.Koenigii*. These pesticides may be used for the control of other insect pests accordingly. The side effects of the chemical control must always be kept in mind and least mammalian and environmental toxicity must be ensured.

V. CONCLUSION

The three pesticides differ in the level of toxicity in the order of: Chlorpyrifos > λ-Cyhalothrin > Neem extract.

Effect on biochemical parameters of GOT and protein content is different.

Survivorship and fecundity are adversely affected by the three pesticides at different rates.

Survivorship data of *D. koenigii* may help in drawing of life tables and assessment of the complete demography of the said pest.

The determined LC50 values will ensure control and safety aspects of the pesticides.

The study can be enhanced to control other insect pests by the given control agents ensuring least mammalian toxicity and environmental persistence.

Botanical pesticide i.e. Neem extract can be used effectively in various ways for the control of insect pests without harming the natural enemies and with no resistance promoting problems.

REFERENCES

- [1]. Afzal, M., Rehman, S.U. and Siddiqui, M.T., 2009. Appearance and management of a new devastating pest of cotton *Phenacoccus solenopsis* Tinsley, in Pakistan. Beltwise cotton conference San Antonio, Texas, pp 5-8.
- [2]. Ahmad, N., Khan, M.H., Toufique, M. and Rauf, I., 2011. Insect pests management of bt cotton through the manipulation of different eco-friendly techniques. *The Nucleus* 48(3): 249-254.
- [3]. Al-Deeb, M.A., Wild, G.E. and Zhu, K.Y., 2001. Effects of insecticides used on corn, sorghum and alfalfa for the predator *Orius insidiosus* (Hemiptera: Anthocoridae). *J. Eco. Entomol.* 94(6): 1353-1360.
- [4]. Ali, M. and Masarrat, J., 2005. Comparative studies of toxicological effects of Coopex and Lambda Cyhalothrin on choline esterase activity and total protein contents in the adult of stored grain pests. Unpublished M.Sc. Thesis.
- [5]. Anonymous, 2007. Pakistan cotton statistics, Directorate of Marketing and Economic Research, Pakistan Central Cotton Committee, Min. Food, Agric. and Livestock Govt. Pakistan.
- [6]. Anonymous, 2010. Agricultural Statistics of Pakistan, Govt. Pak., Min. Food, Agric. (Economic wing), Islamabad, Pakistan. 29-30.
- [7]. Arias, R.D., Lehane, M.J., Schofield, C.J. and Fournet, A., 2003. Comparative evaluation of pyrethroid insecticide formulations against *Triatoma infestans* (Klug) residual efficacy for substance. *Mem. Inst. Oswaldo Cruzout.*, 98(7): 975-980.
- [8]. Berg, G.L., (ed) 1986. Farm Chemical Hand Book. Willoughby, OH: *Meister Publishing Company*.
- [9]. Fakhri, M., Alam, S., Shafiq, A.M., Khowaja, J. and Haider, A., 2011. Comparative assessment of conventional and non-conventional insecticides on the biology of *D. koenigii* Fabr., *J. Ent. Res.*, 35(3): 209-214.
- [10]. Frazer, H.L., 1994. Observation on the method of transmission of internal boll disease of cotton by the cotton strainer bug. *Ann. Appl. Biol.*, 31: 271-290.

- [11]. Gatti, S.S., Henderson, G., Abdel-Aal, Y.A.I. and Ibrahim, S.A., 2002. Acetyl choline esterase mediated susceptibility of soldiers and workers of Formosan subterranean termite (Isoptera: Rhinotermitidae) to chlorpyrifos. *J. Eco. Entomol.* 95(4): 813-819.
- [12]. Garcia, J.F., Grisoto, E., Vendramin, J.D. and Bothelho, P.M., 2006. Bioactivity of neem, *Azadiracta indica* against spittle bug, *Machanarva fimbriolata* (Hemiptera: Cercopidae) on sugarcane. *J. Eco. Entomol.*, 95(4): 813-819.
- [13]. Kidds, H. and James, D.R., 1991. The Agrochemical Handbook, 3rd Edition, Royal Society of Chemistry Information Service, Cambridge, UK, 2-13.
- [14]. Kodandaram, M.H., Azad, Thakur, N.S. and Shylesha, A.N., 2008. Toxicity and morphogenetic effects of different botanicals on red cotton bug *D. koenigii* Fab. (Hemiptera: Pyrrhocoridae) in North Eastern Hill (NEH) region of India. *J. Biopest.*, 1(2): 187-189.
- [15]. Koul, O. and Wahab, S., (eds. 2004), *Neem: Today and in the New Millennium*. Kluwer, Dordrecht, The Netherlands.
- [16]. Krattiger, A., 1997. Insect resistance in crops: A case study of *Bacillus thuringiensis* (Bt) and its transfer to developing countries. ISAAA Briefs, 2: 42.
- [17]. Meister, R.T., 1992. *Farm chemicals Handbook*. Meister Publishing Company. Willoughby, OH.
- [18]. Naqvi, S.N.H. and Ahmad, M.D., 2000. The efficacy of a phytopesticide in comparison with perfection against sucking pests of Pakistan. *Turk. J. Zool.*, 24: 403-408.
- [19]. Nathan, S.S., Choi, M.Y., Paik, C.H., Seo, H.Y., Kim, J.D. and Kang, S.M., 2007. The toxic effects of neem extract and azadirachtin on the brown plant hopper, *Nilaparvata lugens*. *Chemosphere*. 67(1): 80-88.
- [20]. Pearson, E.O., 1958. *The insect pest in Tropical Africa*, London. Common Wealth Institute of Entomology, 355pp.
- [21]. Prenachandra, D.W.T.S., Borgemeister, C. and Poehling, H-M., 2005. Effect of neem and sphinose on *Ceratothripoides claratris* (Thysanoptera: Thripidae). *J. Ecol. Entomol.* 98(2): 438-448.
- [22]. Rakotondravelo, M.L., Anderson, T.D., Charlton, R.E. and Zhu, K.Y., 2006. Sublethal effects of three pesticides on larval survivorship, growth and macromolecular production in the aquatic midges, *Chironomas tentans* (Diptera: Chironomidae). *J. Archiv. Env. Contam. and Toxicol.* 51(3): 352-359.
- [23]. Ranga Rao, G.V., Visalakshmi, V., Suganthi, M., Reddy, P.V., Reddy, Y.V.R. and Rameshwar Rao, V., *Int. J. Trop. Insect. Sc.*, 27(3/4): 229-225.
- [24]. Roger, C., Vincent, C. and Coderre, D., 2009. Mortality and predation efficacy of *Coleomegilla maculate lengii* Timb. Following application of neem extracts. *J. Appl. Entomol.* 119 (1-5): 439-440.
- [25]. Saeed, S., Ahmad, M. and Kawon, Y.J., 2007. Insecticidal control of the mealy bug *Phenacoccus gossypiphilous*. *J. Entomol. Res.* 37(2): 76-80.
- [26]. Saegraves, M.P. and Robert, M.Mc Pherson, 2003. Residual susceptibility of the red imported Fire Ant (Hymenoptera: Formicidae) to four agricultural insecticides. *J. Econ. Entomol.*, 96(3): 645-648.
- [27]. Sahito, H.A., Abro, G.H., Syed, T.S., Memon, S.A., Mal, B. and Kaleri, S., 2011. Screening of pesticides against cotton mealy bug *Phenacoccus solenopsis* Tinsley and its natural enemies on cotton crop. *Int. Res. J. Biochem. Bioinform.*, 1(9): 232-236.
- [28]. Schaefer, C.W. and Ahmad, I. 2000. Cotton strainers and their relatives (Pyrrhocoridae and Largidae). *Heteroptera of Economic Importance*. Boca Raton (eds): CRC Press, 271-307.
- [29]. Shabozoi, N.U.K., Abro, G.H., Syed, T.S. and Awan, M.S., 2011. Economic appraisal of pest management options in okra. *Pakistan J. Zool.*, 43(5): 869-878.
- [30]. Snodgrass, G.L., Adamezyk, J.J.Jr. and Gore, J., 2005. Toxicity of insecticide in a glass vial bioassay to adult Brown, Green and Southern Green Stink Bugs (Heteroptera: Pentatomidae). *J. Eco. Entomol.* 98(1): 177-181.
- [31]. Sprekel, R.K., 2000. *Cotton plant and pest monitoring manual for Florida*, Florida. <http://ifas.ufl.edu/NFREC> (09 March 2005).
- [32]. Tabassum, R., Jahan, M., Azmi, M.A., Naqvi, S.N.H. and Ahmad, I., 1992. Determination of toxicity of Methoprene and Neem formulations against stored grain pest, *Sitophilus oryzae* L. *Pak. J. Pharm. Sci.*, 5(2): 167-174.
- [33]. Tabassum, R., Jahan, M. and Naqvi, S.N.H., 1994. Determination of toxicity of Malathion and RB (a formulation of Neem extract) against *Tribolium castaneum* adults and their effects on transaminases. *Neem Newsletter*. 11(1): 7-9.
- [34]. Tabassum, R., Jahan, M., Nurulain, S.M. and Naqvi, S.N.H., 1998. Determination of toxicity of Fenprothrin (Pyrethroid) and neem formulation (RB-a+PBO+TX-100) and its effects on transaminases (GOT and GPT) against *Alphitobios diaperinus* adults. *Turk. J. Zool.*, 22(4): 319-322.
- [35]. U.S. Environmental Protection Agency. 1986(Sept). *Ambient water quality criteria for chlorpyrifos-1986*. Office of Water Regulation and Standard. Criteria and Standard Division, Washington, D.C.
- [36]. Vandekerkhove, B. and De Clercq, P., 2004. Effect of an encapsulated formulation of Lambda Cyhalothrin on *Nezara viridula* and its predator *Podisus maculiventris*. *Florida Entomol.*, 84(2): 112-118.
- [37]. Weathersbee, A.A., Meisch, M.V., Inman, A. and Dame, D.A., 1991. Activity of Lambda Cyhalothrin applied as an ultra low volume ground treatment against *Anopheles quadrimaculatus*. *J. Am. Mosq. Contr. Assoc.*, 7(2): 238-241.
- [38]. World Health Organisation. *Cyhalothrin, Environmental Health Criteria*, 99. Geneva, Switzerland, 1990.